linearized by *Not* I using T4 DNA ~~liagse~~ ligase resulting in a pPIC9K/P4Hα expression vector. The direction of insert was identified.

Change(s) applied to document, /S.G.L./ 4/13/2011

Please amend the paragraph beginning at page 38, line 16, as follows:

3) P4Hβ cDNA cloing and recombinant expression vector construction

According to the known chicken P4Hβ subunit gene sequence, the primers F:5'GCG GCC GCA CAG CCC CTG GAG GAG 3' (SEQ ID No.24) and R: 5'GCG GCC GCG GTG ATG TAG ATC AGT C 3' (SEQ ID No.25) were designed and *Not* I restriction sites were introduced. RNA was extracted from sternum of 17-day-old chicken embryos followed by RT-PCR. 1.5 mM [Mg^{2+}] was used in the PCR system.

Please amend the paragraph beginning at page 39, line 20, as follows:

PCR was performed using the plasmids pGEM-T/P4Hα and pGEM-T/P4Hβ in Example 8 and 9, as templates. The primers' sequences were P4Hα: F: 5'GCGGCCGC GAT ACT GCT ACG AAA G3' (SEQ ID No.26); R: 5'GCGGCCGC CTC CAA CTC TGA TAA C 3' (SEQ ID No.27); P4Hβ: F: 5'GCGGCCGC CAG CCC CTG GAG GAG -3 (SEQ ID No.28)'; and R: 5' GCGGCCGC TTA ATC ATC ATC AGC 3' (SEQ ID No.29).

Please amend the paragraph beginning at page 40, line 14, as follows:

Since the P4Hβ expression unit was located upstream, PCR was used to examine whether the direction of P4Hα and β was coincident. The upstream primer (5' GCGGCCGC CAG CCC CTG GAG GAG 3') (SEQ ID No.28) and downstream primer (5' GCGGCCGC CTC CAA CTC TGA TAA C 3') (SEQ ID No.27) for amplifying

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4/13/2011

Please amend the paragraph beginning at page 33, line 12, as follows:

1) The construction of the eukaryotic expression vector pPICZαB/ CCOL2A1

The eukaryotic expression vector was constructed as follows: PCR was performed using the primers (sequence 25 5'GGT ACC TTG GTG GAA ACT TTG CGG 3'; SEQ ID No.18) and (sequence 26 5'GGT ACC GTT ACA AGA AGC AGA CTG 3'; SEQ ID No.19), and pGEM-T/CCOL2A1 as template. The PCR mixture contained 25 μl of 2×GC Buffer I, 4 μl of dNTP (2.5 mM), 0.5 μl of 20 μM primers.

Please amend the paragraph beginning at page 37, line 21, as follows:

According to the chicken P4Hα gene sequence, primer a: 5'AGA TAC TGC TAC GAA AGA CCC CGA G 3' (SEQ ID No.20) and b: 5' CTC TCT TGG TTG TAG CCC TCA TCT G 3' (SEQ ID No.21) were designed. The PCR system contained 5 μl of 10×PCR Buffer, 5 μl of 25 mM $MgCl_2$, 4 μl of 2.5 mM dNTP Mix, 0.5 μl of 20 μM primers, 1μl of cDNA ,0.5 μl Taq, and water to a final volume of 50 μl. PCR products were examined by 1% agarose electrophoresis.

Please amend the paragraph beginning at page 38, line 7, as follows:

The P4Hα PCR product described above was ligated with pGEM-T, resulting in a pGEM-T/P4Hα plasmid. A *Not* I restriction site was introduced by PCR. The primers a: 5'GCG GCC GCA GAT ACT GCT ACG AAA G 3' (SEQ ID No.22) and b: 5'GCG GCC GCC TCT CTT GGT TGT AGG 3' (SEQ ID No.23), and Pfu DNA polymerase were used for PCR. PCR products were given polyA tails then ligated with a pGEM-T vector and sequenced. The target fragments were recovered after the pGEM-T/P4Hα was digested by *Not* I. The P4Hα gene fragment was ligated with the pPIC9K vector